

Topical Treatment of Vaccinia Virus Infection with an Interferon Inducer in Rabbits

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Ointment containing 1.7 mg/g of the interferon inducer poly (ICLC) (a complex of polyriboinosinic-polyribocytidylic acid with poly-L-lysine and carboxymethylcellulose) was effective both prophylactically and therapeutically against vaccinia virus infection in rabbit skin. Poly (ICLC)-treated animals developed higher titers of local interferon and of circulating virus-neutralizing antibodies than placebo-treated animals. Serum levels of >100 units of interferon/ml were found 24 hr after application of the poly (ICLC) ointment to the skin of uninfected rabbits.

The complex of polyriboinosinic-polyribocytidylic acid [poly (I)•poly (C)] with poly-L-lysine and carboxymethylcellulose [poly (ICLC)] has been shown to be effective systematically as an interferon inducer in rodents, monkeys, and chimpanzees [1] and in humans [2]. In nonhuman primates it has controlled simian hemorrhagic fever [3], street virus rabies [4], and yellow fever [5] and has some therapeutic effect in chimpanzees with chronic hepatitis B infection [6].

We have found that an ointment containing this complex is effective both prophylactically and therapeutically against infections due to vaccinia virus in the skin of rabbits. The present report summarizes the results of this study.

Materials and Methods

Poly (ICLC). Poly (ICLC) was prepared as described previously [1]. The poly (ICLC) solution contained 2 mg of poly (I)•poly (C)/ml. One part of Aquaphor ointment base (Duke Laboratories, Norwich, Conn.) was mixed with six parts of poly (ICLC) solution, and this mixture yielded a white ointment containing ~ 1.7 mg of poly (I)•poly (C) equivalent/g. A control (placebo) ointment was made comparably, with use of 0.5% carboxymethylcellulose instead

of poly (ICLC). Previous studies showed that poly-L-lysine in carboxymethylcellulose neither induced interferon nor had any antiviral action in vivo (H. B. Levy, unpublished observations).

Virus. The IHD-E strain of vaccinia virus was propagated in HeLa cells. The stock virus preparation contained 10^7 skin lesion doses/ml. Rabbits were injected intracutaneously with 10^3 skin lesion doses in 0.1 ml of a virus dilution.

Interferon assay. Interferon was assayed in secondary rabbit kidney cells, on 96-well microtiter plates (Falcon Plastics, Oxnard, Calif.) by a CPE protection method, with use of vesicular stomatitis virus as the challenge organism. Results were expressed as reference units/ml, with use of a standard rabbit interferon as a reference. The standard reagent was the NIAID (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.) reference reagent no. G-019-901-028 (1975-1977 catalogue), with a titer of $4.3 \log_{10}$ units/ml.

Antibody assay. Vaccinia virus (100 pfu) was incubated with serial twofold dilutions of rabbit serum for 1 hr at 37 C. The virus was then added to tube cultures of HR-203 cells (HEM Research, Inc., Rockville, Md.). The reciprocal of the highest dilution of serum that reduced CPE by half was considered to be the titer of neutralizing antibody in serum.

Results

~~A first series of~~ experiments was designed to test whether poly (ICLC) ointment would be

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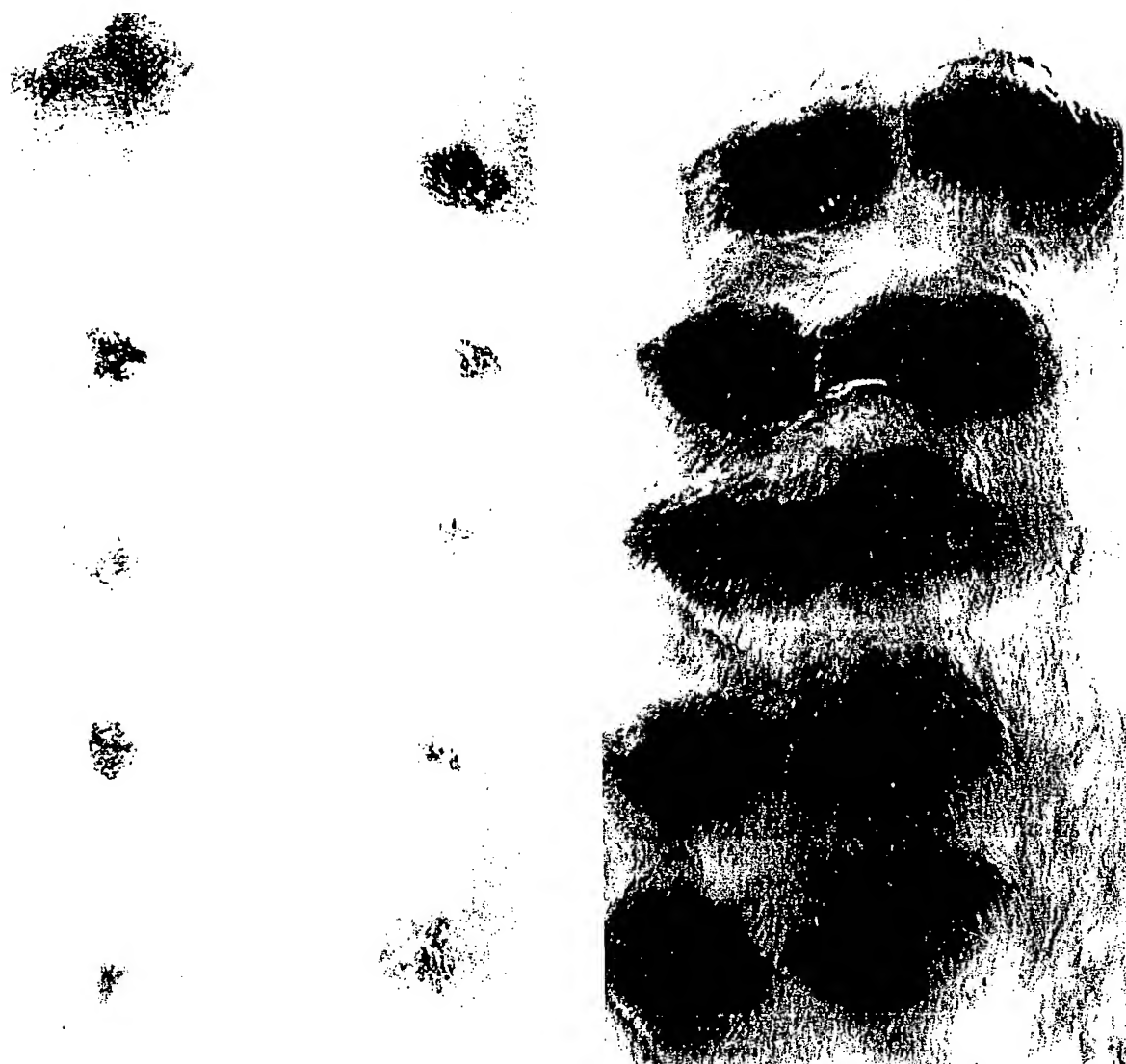


Figure 1. The effect of treatment with ointment containing either a complex of polyribonucleosinic-polyribonucleotidylic acid, poly-L-lysine, and carboxymethylcellulose (*left*) or placebo (*right*), applied after challenge with vaccinia virus, on the development of lesions, as observed in rabbits seven days after infection. See Materials and Methods for preparation of the ointments and Results for treatment schedule ($\times 1.5$).

effective prophylactically after topical application to rabbit skin. In these experiments an area of ~ 2 inches \times 5 inches on the backs of rabbits was shaved, and ~ 1 g of ointment containing 1.7 mg of poly (ICLC) was rubbed into the shaved area. After 8 hr vaccinia virus was injected intracutaneously into 10 separate sites (total, 10^4 skin lesion doses per rabbit). Treatment with ointment was repeated 24, 48, 72, and 96 hr after the first treatment. Control rabbits were treated

with the placebo ointment. Four experiments of this type were performed, with a total of eight poly (ICLC)-treated and eight placebo-treated animals. Similar results were obtained in all four experiments. Treatment of the skin before infection and continuing after infection materially decreased the progression, as well as the number, of lesions. Animals receiving the placebo ointment developed lesions beginning about three days after infection and becoming severe

by day 6. Three of the eight control animals developed encephalitis and died, with vaccinia virus present in the brain. Lesions in poly (ICLC)-treated animals rarely progressed beyond 1-3 mm in diameter, were slightly elevated, and were pink. There was no systemic disease in treated animals.

Subsequent experiments were performed to determine whether poly (ICLC) ointment would be effective when treatment was begun just after the lesions became visible, about three days after infection, with additional daily treatment for three days. Four experiments, each with two poly (ICLC)-treated and two placebo-treated rabbits, were performed. Treatment stopped further development of the lesions in seven of the eight poly (ICLC)-treated animals. Lesions became very severe in all of the eight animals treated with the placebo ointment. Lesions in animals of both groups are shown in figure 1.

In another group of four poly (ICLC)- and four placebo-treated animals infected intracutaneously with vaccinia virus, titers of interferon and of virus in the skin were determined. Treatment began three days after infection and was continued for three additional days. The skin samples were taken seven days after infection, 8 hr after the last treatment. Data indicated that more interferon and less virus were present in infected areas from poly (ICLC)-treated than from placebo-treated animals (range of interferon titers, 400-800 units/ml vs. 30-100 units/ml, respectively; viral titers, 4.5×10^1 vs. 5×10^4 pfu/g of wet tissue, respectively).

For the antibody assays samples of blood were taken from six poly (ICLC)- and six placebo-treated rabbits 10 days after infection. The mean titer of virus-neutralizing antibody was higher in the poly (ICLC)-treated animals (1,500, with a range of 600-1,700) than in the placebo-treated animals (160, with a range of 80-300).

Putting the inducer-containing ointment on the shaved skin of uninfected rabbits led to the development of a significant level of circulating interferon. Figure 2 shows the representative kinetics of such interferon production. That the antiviral activity in the serum is attributable to interferon and not to residual poly (ICLC) is suggested by the observation that the interferon-containing rabbit serum does not induce viral

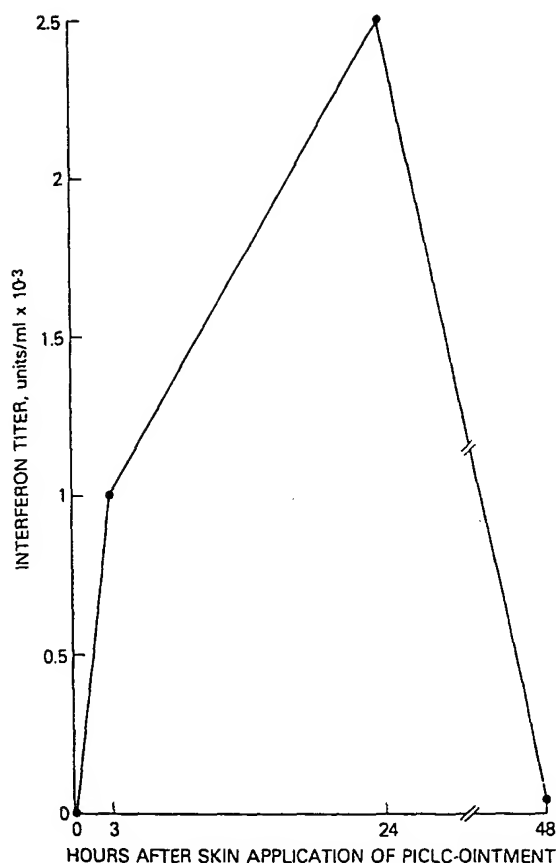


Figure 2. Mean titers of interferon in serum from four uninfected rabbits after topical application of 1.5 g of ointment containing 1.7 mg of polyribonucleoside-polyribocytidylic acid [poly (I) · poly (C)] equivalent/g of ointment. PICLC = a complex containing poly (I) · poly (C), poly-L-lysine, and carboxymethylcellulose.

resistance in human or rat cells that are sensitive to poly (ICLC).

Discussion

The data presented here demonstrate that a superficial viral infection can be controlled by the topical application of an interferon inducer. Associated with this control is the production of significant quantities of interferon in the skin. Despite the decrease in virus, there was an increase in the level of neutralizing antibody in serum, but the differences might be attributable to a difference in the kinetics of antibody production between the control and poly (ICLC)-treated groups. The increase in antibody is, how-

ever, in accord with the observation that poly (ICLC) is a potent immune adjuvant in mice, rats, and monkeys when used in conjunction with an inactivated Venezuelan equine encephalitis virus vaccine [7] and with a split-product swine influenza virus vaccine [8].

Vaccinia virus lesions are of decreasing importance in human disease today. Genital herpesvirus infections, on the other hand, are currently serious problems. Although the vaccinia virus infection induced in this study was very severe, probably more severe than that encountered in naturally acquired infection, vaccinia virus is more sensitive to interferon than is herpesvirus. Whether poly (ICLC) will be useful in genital herpesvirus infection in humans is the subject of an ongoing study.

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